

$NF-\kappa B$ oscillations and cell-to-cell variability

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Abstract

Oscillations in the transcriptional activator $NF-\kappa B$ localized in the nucleus have been observed when a cell is stimulated by an external agent. A negative feedback based on the protein $I\kappa B$ whose expression is controlled by $NF-\kappa B$ is known to be responsible for these oscillations. We study $NF-\kappa B$ oscillations, which have been observed both for cell populations by Hoffmann et al. (2002) and for single cells by Nelson et al. (2004). In order to study cell-to-cell variability we use Gillespie's algorithm, applied to a simplified version of the model proposed by Hoffmann et al. (2002). We consider the amounts of cellular $NF-\kappa B$ and activated IKK as external parameters. When these are fixed, we show that intrinsic fluctuations are small in a model with strong transcription, as is the case of the Hoffmann et al. (2002) model, whether transcription is quadratic or linear in the number of $NF-\kappa B$ molecules. Intrinsic fluctuations can however be large when transcription is weak, as we illustrate in a model variant. The effect of extrinsic fluctuations can be significant: cell-to-cell fluctuations of the initial amount of cellular $NF-\kappa B$ affect mainly the amplitude of nuclear $NF-\kappa B$ oscillations, at least when transcription is linear in the number of $NF-\kappa B$ molecules, while fluctuations in the amount of activated IKK affect both their amplitude and period, whatever the mode of transcription. In this case model results are in qualitative agreement with the considerable cell-to-cell variability of $NF-\kappa B$ oscillations observed by Nelson et al. (2004).

Keywords: cell-to-cell variability, $NF-\kappa B$ oscillations, fluctuations, Gillespie's algorithm

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1 Introduction

For a number of biological processes cell-to-cell variability can be very large, even for clonal populations, as pointed out by Dower and Qvarnstrom (Dower and Qvarnstrom, 2003), who studied immune response signaling systems connected to the family of TIR (Toll/interleukin-1) receptors. In such cases average cell behavior is a poor indicator of the way individual cells respond. When for example cell expression as a function of inducer or enhancer is stochastic, graded average response can hide all or nothing single cell response, as shown in a number of studies (Fiering et al., 1990, 2000; Hume, 2000; Ko, 1992; Nair et al., 2004). It is therefore important that in both experiment and modeling cell-to-cell variability be investigated. In particular, one needs to be attentive to this fact when modeling a biological system with a set of chemical rate equations for concentrations: these by definition encapsulate average behavior only. A case in point is provided by recent experiments and modeling of $NF-\kappa B$ activation as a result of $TNF\alpha$ stimulation of human Jurkat T cells, U937 monocytes, and mouse fibroblasts (Hoffmann et al., 2002), or human cervical carcinoma and type-S neuroblastoma cells (Nelson et al., 2004). Hoffmann et al. (2002) measure average cell behavior and model it with a set of chemical rate equations for concentrations, whereas Nelson et al. (2004) examine the same model but give experimental results on single cells only, some of which show large cell-to-cell variations. It is difficult to reconcile well defined average oscillatory behavior with cell-to-cell variability in both amplitude and period of oscillations. Our aim is to understand (within the confines of the model of Hoffmann et al. (2002)) the origin of the observed (Nelson et al., 2004) cell-to-cell fluctuations of nuclear $NF-\kappa B$ oscillations.

The fluctuations which underlie cell-to-cell variability can be separated into intrinsic and extrinsic (Elowitz et al., 2002; Raser and O'Shea, 2004). Intrinsic fluctuations are those which result from random occurrence of a defined set of reactions at random times, leading to cell-to-cell variability for genetically identical cells in identical, fixed environments. Extrinsic fluctuations can have multiple origins, such as fluctuations in cytoplasmic and nuclear volumes, in the initial state of the cell, signaling cascade fluctuations, or variations in the number of regulatory molecules.

We should point out that $NF-\kappa B$ oscillations are a transient phenomenon, unlike the oscillatory process involved in circadian rhythms. Under persistent stimulation of the cell, activation of IKK itself is temporary, either through adaptation (Hoffmann et al., 2002) or through the action of inhibitory factor A20 (Lipniacki et al., 2004). Thus mathematically the cell which is in a steady state before stimulation returns to a steady state some time (many hours) after stimulation. The amplitude and period of oscillations change as time progresses, and they disappear ultimately.

We use stochastic simulations (Gillespie, 1977) of the rate equations and show the following:

- intrinsic fluctuations are very small for the model parameters used in the literature (Hoffmann et al., 2002; Nelson et al., 2004), which correspond to strong transcription, and they affect only late nuclear $NF-\kappa B$ oscillations. Intrinsic fluctuations can however be large if promoter binding is weak, with rate constants for transcription and translation significantly different from those used by Hoffmann et al. (2002)
- extrinsic fluctuations can be significant: fluctuations in the initial amount of activated IKK lead to both amplitude and periodicity fluctuations of $NF-\kappa B$ oscillations, which are features shown by the single cell data of Nelson et al. (2004). This result, for the model parameter set of Hoffmann et al. (2002), is independent of whether transcription is linear or quadratic in the amount of nuclear $NF-\kappa B$. However for fluctuations in the initial amount of cellular $NF-\kappa B$ cell-to-cell variability depends on the mode of transcription: only for quadratic transcription are both amplitude and period affected. For linear transcription only the amplitude of oscillations varies.

These results on cell-to-cell variability make clear the importance of determining experimentally the form of the transcriptional relationship between $NF-\kappa B$ and $I\kappa B\alpha$, and the actual values of transcriptional and translational rate constants.

In the next section we describe the model. This is followed by a section of results. Here we discuss intrinsic noise and study a variant of the Hoffmann et al. (2002) model with a different transcription mechanism in order to gain more insight into the generation of intrinsic noise. We also describe the results of fluctuations in the initial amount of $NF-\kappa B$, and in the number of activated IKK molecules. We conclude with a discussion in which we speculate on the functional role of a threshold in the number of $NF-\kappa B$ oscillations, as cell stimulus, and therefore the amount of activated IKK decreases in intensity.

2 Description of model

$NF-\kappa B$ (nuclear factor κB) plays an important regulatory role in cell immune response and survival (for a review, see Tian and Brasier, 2003). Under normal conditions, it is sequestered in the cell cytoplasm through binding to $I\kappa B$. When the cell is stimulated through for example $TNF\alpha$, the ensuing signaling cascade leads to activation of IKK, which in turn through binding to $NF-\kappa B$ and $I\kappa B$, leads to ubiquitination of $I\kappa B$, and the freeing of $NF-\kappa B$. $NF-\kappa B$ enters the cell nucleus (Carlotti et al., 1999), and regulates the expression of a number of genes, in particular the gene expressing $I\kappa B$. $I\kappa B$ protein enters the nucleus, binds to $NF-\kappa B$, and that complex is exported. Thus $NF-\kappa B$ which initially was sequestered, then released after IKK activation, ends up by being sequestered again. As a result, the concentration of nuclear $NF-\kappa B$ shoots up shortly after stimulation, and then decreases because of the negative feedback associated with $I\kappa B$ expression through $NF-\kappa B$ itself binding to the relevant promoter. The process can repeat itself if the time scales associated with nuclear

import and export, transcription and translation are right. Indeed measurements show that the concentration of nuclear $NF-\kappa B$ oscillates over several hours after the onset of a lasting stimulation (Hoffmann et al., 2002; Nelson et al., 2004).

For the points we wish to make concerning cell-to-cell variability, a pared down version of the model of Hoffmann et al. (2002), which only includes the $I\kappa B$ isoform responsible for $NF-\kappa B$ oscillations, suffices. The original model includes 64 ordinary differential equations for concentrations, with reaction rate constants determined or constrained from experiment, and others constrained by fits to the data obtained with the use of GEPASI software (Mendes, 1993).

There are three isoforms of $I\kappa B$, the main one being $I\kappa B\alpha$, whose transcription depends strongly on the presence of nuclear $NF-\kappa B$. For the other two, transcription is not regulated by $NF-\kappa B$; Hoffmann et al. (2002) were interested in elucidating the role of these other two isoforms in damping $NF-\kappa B$ oscillations. Therefore their system of equations is extensive, each isoform participating in the same set of reactions (except for $NF-\kappa B$ dependent transcription), but without any interaction between isoforms themselves. In our simplified version (the number of reactions is about 1/3 of those of the original model), we will only consider the dominant isoform $I\kappa B\alpha$, which is solely responsible for the occurrence of $NF-\kappa B$ oscillations, and the only one whose knockout is lethal (Gerondakis et al., 1999).

Since we are interested in intrinsic noise issues, a model based on ordinary differential chemical rate equations is inadequate. We use Gillespie's algorithm (Gillespie, 1977, 2001) to study the impact of including both the random occurrence of chemical reactions, and fluctuations in external parameters on the shape of $NF-\kappa B$ oscillations.

We should mention another model of $NF-\kappa B$ oscillations (Lipniacki et al., 2004), which has as its starting point the Hoffmann et al. (2002) model, but focuses in addition on the role of A20, which is regulated by $NF-\kappa B$, and acts as an inhibitor of IKK, itself activated as a result of cell stimulation by an external agent. There is also a discussion of $NF-\kappa B$ oscillations by Monk (2003) in the context of a model of ordinary differential equations with time delays.

The reactions we have used in our model are summarized in Table 1 together with the relevant rate constants, which correspond to the choices made by Nelson et al. (2004, supplementary material)) for the reactions of Hoffmann et al. (2002). Transcription is linear in the number of $NF-\kappa B$ molecules (Lipniacki et al., 2004; Nelson et al., 2005), rather than quadratic as in the original model (Hoffmann et al., 2002). This change, from quadratic to linear, diminishes the sensitivity of $NF-\kappa B$ oscillations to fluctuations in the initial amount of $NF-\kappa B$ present in a cell (Barken et al., 2005; Nelson et al., 2005). We will give results for both choices of transcription in the next section. The model as it stands still has some unsatisfactory features, namely the amount of transcribed molecules rises linearly (or quadratically as in Hoffmann et al. (2002)) with the number of $NF-\kappa B$ molecules entering the cell nucleus, without any mechanism of saturation, and the number of $I\kappa B\alpha$ mRNA molecules is

excessive, of the order of a thousand in the steady state (for the simulation underlying Figure 1), rising to much higher numbers (several times ten thousand) once IKK is activated. We have however checked, as we will discuss in section 3.1., that as a result of decreased transcription and a corresponding increased translation, the number of $I\kappa B\alpha$ mRNA molecules is reduced, while $NF-\kappa B$ oscillations themselves are not affected, because the amount of $NF-\kappa B$ present in the cell is large; the intrinsic noise in the system, however, becomes larger, the more so as the transcription rate decreases.

In the model, $NF-\kappa B$ is not degraded, and therefore the number of $NF-\kappa B$ molecules remains constant through the formation and disintegration of complexes in which it participates. We use cells with volumes of $1000 \mu^3$, divided equally between cytoplasm and nucleus. We take an amount of $0.05 \mu M$ relative to the total cell volume for $NF-\kappa B$. Thus the number of $NF-\kappa B$ molecules initially introduced, corresponding to $0.05 \mu M$ for the whole cell, is 30,000. After an equilibration period of 3000 minutes, we follow Hoffmann et al. (2002) in introducing an equal amount of IKK ($0.05 \mu M$), which represents activation of the signaling pathway through cell exposure to $TNF\alpha$.

The shape of $NF-\kappa B$ oscillations will for a given cell volume depend on (all other parameters being fixed) the fraction of volume occupied by the nucleus (Lipniacki et al., 2004), because reactions take place in both cytoplasm and nucleus and there is nuclear import and export. Their shape depends as well on the comparative amount of initial $NF-\kappa B$ and IKK activated after cell stimulation.

$NF-\kappa B$ oscillations, which are more or less damped, are sensitive to a number of model parameters such as the rate of transcription of $I\kappa B\alpha$ or its translation, or $I\kappa B\alpha$ mRNA degradation, which are unknown and determined from fits to experiments. Thus it is not always possible to evaluate the significance of the precise form of the oscillations from comparing model results with experiment.

3 Results.

Data are obtained in different cell lines (Hoffmann et al., 2002; Nelson et al., 2004) with different techniques and therefore comparisons can be hazardous. Nevertheless, the data on $NF-\kappa B$ signaling show well defined oscillations (of period close to 100 minutes) when averaged over cells, when only isoform $I\kappa B\alpha$ is present (Hoffmann et al., 2002, supplementary material, fig. S1). However the single cell data of Nelson et al. (2004) show large cell-to-cell variations in both amplitude and periodicity, which if typical, would be expected to broaden or even wash out average $NF-\kappa B$ oscillations. It is in this context that we will next discuss our results. We will examine three possible sources of cell-to-cell variability: intrinsic fluctuations on one hand, and on the other extrinsic fluctuations in either the initial number of $NF-\kappa B$ molecules or of activated IKK molecules.

3.1 Intrinsic noise.

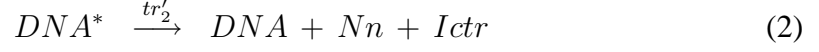
Intrinsic noise is small in the models of Hoffmann et al. (2002) and Nelson et al. (2004) given the rate constants they employ. Whether transcription is linear or quadratic in the number of $NF-\kappa B$ molecules, intrinsic fluctuations become significant only about 600 minutes after IKK activation. In Figure 1 we show, for the case of linear transcription, and for the parameters of Table 1, nuclear $NF-\kappa B$ oscillations as a function of time after activation by IKK after 3000 minutes of equilibration. For this figure the initial numbers of $NF-\kappa B$ and activated IKK molecules are fixed. Thus any fluctuation is purely intrinsic, due to the random order in which reactions occur in any given cell. The first three or four oscillations have a period of about 100 minutes, thereafter the period lengthens. The figure shows average behavior and the results for three randomly chosen single cells. The comparison illustrates the point that fluctuations are very small: in each cell oscillations for 8 hours after activation are almost identical to the average. (The rapid small amplitude fluctuations over times less or much less than a minute are washed out in the representation of Figure 1.) Thus cell-to-cell variability is very small. The main reason is that the number of molecules is large: with values of the order of $0.05 \mu M$ for initial numbers of $NF-\kappa B$ and activated IKK molecules, and cells of volume $1000 \mu^3$, their numbers are in the tens of thousands. Moreover transcription, the strength of which increases with the number of $NF-\kappa B$ molecules, is strong. If in the models considered, one decreases, while keeping all other rate constants unchanged, the rate of transcription by merely a factor of 2 (for linear transcription) or 4 (for quadratic transcription), $NF-\kappa B$ oscillations beyond the first one are very weak and disappear.

Nevertheless the strength of intrinsic fluctuations indeed varies with the values of the rate constants. As already mentioned in Section 2, we have decreased, within the model with linear transcription, the rate of transcription of $I\kappa B\alpha$ mRNA by a factor of 10, in order to have fewer mRNA, and increased translation by the same factor, thus keeping the number of $I\kappa B\alpha$ proteins essentially unchanged. This model still yields good oscillations in nuclear $NF-\kappa B$ but with increased cell-to-cell variability, as shown in Figure 2, and as compared to the single cell curves in Figure 1. The decreased copy number of $I\kappa B\alpha$ mRNA thus leads to noise in its own oscillations that is reflected in the behavior of $NF-\kappa B$. The intrinsic noise is increased (up to factors of two) over the original model. We will investigate further the connection between strength of transcription and intrinsic noise in the following section with a more appropriate model which includes explicit promoter binding.

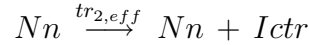
3.1.1 Model variant with DNA.

The model of Hoffmann et al. (2002) treats transcription in an oversimplified way without including DNA. In order to examine the effect of the stochastic binding and unbinding of $NF-\kappa B$ to the promoter site of the DNA on the level of intrinsic noise we have considered

the following model: the transcription of the mRNA coding for $I\kappa B\alpha$, denoted by $Ictr$, occurs through the reactions.



Since the sum of the concentrations of the DNA and the complex DNA^* is conserved, we have, $c_D + c_{D^*} = c_0$ where $c_0 = 2/300000 \mu M$ given the volume of the nucleus ($500 \mu^3$) and assuming a diploid cell. If the first reaction above occurs rapidly and is hence, in equilibrium, we can combine the two equations in the spirit of Michaelis-Menten to obtain



where the effective transcription rate that depends on the nuclear $NF\kappa B$ concentration Nn is given by

$$tr_{2,eff} = \frac{c_0 tr'_2}{\frac{kb}{kf} + Nn}.$$

Since the maximum value of Nn is $0.1 \mu M$ we chose $kb/kf = 0.2 \mu M$; changing the value up to $1.0 \mu M$ had negligible effect on the results given below. In order to obtain similar transcription rates as for the linear model we chose $tr'_2 \times c_0$ to be equal to the value of tr_2 used in the original model given in Table 1. In order to ensure that the binding and unbinding of Nn occur sufficiently rapidly we chose $kb = 1200 min^{-1}$ and the translation rate $tr1$ is increased by a factor of 2 from the value given in Table 1. With this high rate of promoter binding, one is in a situation, as for the models of linear or quadratic transcription (Hoffmann et al., 2002; Nelson et al., 2004), where the relevant promoter is strongly activated and transcriptional stochasticity is small (Raser and O'Shea, 2004). This is confirmed by the simulation reported in Figure 3, which shows the concentration and standard deviation of nuclear $NF-\kappa B$ as a function of time after activation by IKK after 3000 minutes of equilibration. The noise is small in the first four oscillations and increases at larger times, in the same way as shown in Figure 1 for linear transcription with the parameter set of Table 1.

Transcriptional noise.

Moving away from the regime of strong transcription embodied in the models with linear or quadratic transcription (Hoffmann et al., 2002; Nelson et al., 2004), one can now explore what happens when promoter binding gets weaker, with a rate that gets closer to the rates which determine $I\kappa B\alpha$ protein ubiquitination, namely rates $r1$ and $r4$ (cf. Table 1). Transcriptional fluctuations are then no longer averaged out over the corresponding time scales (Lewis, 2003). One can do so since, contrary to most rate constants which are determined or constrained by experiment (Hoffmann et al., supp. material, 2002), those of transcription and translation, $tr2$ and $tr1$ respectively (cf. Table 1), result from fits to the oscillation data. However, as one decreases the rate of promoter binding, it is necessary to increase correspondingly

the rate of translation in order to maintain well defined nuclear $NF-\kappa B$ oscillations. Figure 4 illustrates the impact of weak transcription on the strength of intrinsic noise, for a binding rate of $kb = 12min^{-1}$, a hundredfold decrease as compared to the case of strong transcription (see above), and a corresponding 200 fold increase of the translation rate $tr1$ of Table 1. The intrinsic noise is much more significant than in Figure 3, and, as shown in Figure 5, it affects both the amplitude and periodicity of $NF-\kappa B$ oscillations. Indeed, the intrinsic noise is sufficiently large that the oscillations obtained from averaging the stochastic simulations is more damped than those obtained from the deterministic system. This underscores the potential pitfalls of deducing rate constants by fitting with deterministic models.

One could investigate the effects of further reducing promoter binding. For our purpose which is to establish and clarify the possible sources of the observed cell-to-cell variability (Nelson et al., 2004) of $NF-\kappa B$ oscillations, it is sufficient to emphasize that intrinsic noise can be significant, as important as extrinsic noise, if we do not confine ourselves to the fitted values of transcription and translation rates of the set of reactions considered by Hoffmann et al. (2002).

3.2 Extrinsic noise.

Since the data indicate large cell-to-cell fluctuations (Nelson et al., 2004), these must arise from fluctuations in external parameters, at least for the models with strong transcription, for which, as shown above, intrinsic noise is small. We consider two possible sources here, the number of initial $NF-\kappa B$ present in a given cell, which varies between cells in an analogous situation (Dower and Qwarnstrom, 2003), and the amount of IKK , which can vary due to signaling cascade fluctuations. The models considered are those with transcription quadratic in the number of $NF-\kappa B$ molecules (Hoffmann et al., 2002) and its version with linear transcription (Nelson et al., 2004) with the rate constants of Table 1. For quadratic transcription, the linear transcription reaction in Table 1 is replaced by $2Nn \rightarrow 2Nn + Ictr$ with rate constant $tr2 = 1.027125\mu M^{-1}min^{-1}$. For Figure 7 moreover rate constants $tr1$ and $tr3$ of Table 1 are multiplied by 2.

3.2.1 $NF-\kappa B$ fluctuations.

As alluded to in section 2, there is a significant difference here between the models with linear and quadratic transcription (Barken et al., 2005; Nelson et al., 2005). For linear transcription, moderate fluctuations in the amount of $NF-\kappa B$ (see below) affect the amplitude only of single cell oscillations, thus maintaining well-defined average oscillations. For quadratic transcription, both amplitude and period of single cells fluctuate, leading to reduced or washed out average oscillations. The following discussion explains this point.

In Figure 6, for linear transcription, are shown results for $NF-\kappa B$ oscillations for the

average over many cells for a Gaussian distribution of initial number of $NF-\kappa B$ (with average 30000, $\sigma = 5000$) (black curve) and single cell behavior when initial $NF-\kappa B$ is one or two σ away from the average. IKK remains fixed in all cases at $IKK=30000$. Average $NF-\kappa B$ oscillations in Figure 6 are of course very similar to those in Figure 1, diverging from them only beyond the fourth oscillation, when amplitudes become smaller. As expected, single cell behavior differs from the average only in amplitude, significantly so for 2σ deviations from average. But periodicity is maintained for 8 hours after IKK activation, and thus, insofar as the data of Nelson et al. (2004) show significant early variation in oscillation period from cell to cell, there have to be other sources of variability in a model with strong *linear* transcription.

On the contrary, for quadratic transcription, fluctuations in the initial number of $NF-\kappa B$ molecules, affect both amplitude and periodicity of oscillations, not just their amplitude as in the case of linear transcription. The result is that contrary to what happens in Figure 6 for linear transcription, where oscillations remain clear and distinct, for an initial Gaussian distribution of $NF-\kappa B$ over cells (at a fixed number of activated IKK), here oscillations in the number of nuclear $NF-\kappa B$ oscillations, are very much damped out as shown in Figure 7, as a result of cell-to-cell variability in both amplitude and period of oscillations.

3.2.2 IKK fluctuations.

Within the model parameters considered, we turn to studying fluctuations in the initial number of IKK which activates the cellular oscillatory response. Whatever the type of transcription, linear or quadratic, fluctuations in the initial amount of activated IKK lead to strong cell-to-cell variability. We show here our results for the case of linear transcription.

Figure 8 shows, for fixed $NF-\kappa B = 30000$, the average over many cells for a Gaussian distribution of the initial number of IKK (average=30000, $\sigma = 5000$), and single cell response for $IKK=25000$ and $IKK=35000$. Several points can be made here:

- when the initial number of IKK activated by $TNF\alpha$ fluctuates, not only does the amplitude of $NF-\kappa B$ oscillations vary, but also their periodicity
- as a result, single cell fluctuations are washed out in the average. Average response over the cell population represents poorly single cell response beyond the first oscillation
- the difference between maxima and minima of the oscillations gets larger as IKK decreases from its average, while at the same time oscillatory period increases. Possible implications of the latter result are given in the discussion.

It is clear from the figures that whatever the source of cellular fluctuations, cell-to-cell variability typically increases with time after the onset of IKK activation. This can be illustrated further by computing the standard deviation of the noise in the number of nuclear $NF-\kappa B$. In Figure 9 we compare standard deviations as a function of time for the case of Figure 8, when IKK fluctuates according to a Gaussian of average and width of respectively equal 30000 and

5000 molecules, at fixed initial concentration of $NF-\kappa B$ of 30000 molecules ($0.05 \mu M$). On one hand there is $\sigma_{tot}^2 = \overline{\langle Nn^2 \rangle} - \overline{\langle Nn \rangle}^2$, the total variance of nuclear $NF-\kappa B$ oscillations, on the other hand there is $\sigma_{int}^2 = \overline{\langle Nn^2 \rangle} - \overline{\langle Nn \rangle}^2$, the intrinsic variance. Here Nn denotes nuclear $NF-\kappa B$, the brackets denote average over intrinsic fluctuations, and the overline average over fluctuations in the external parameter (Elowitz et al., 2002; Swain et al., 2002). We observe (Fig. 9) that σ_{tot} , which includes extrinsic fluctuations due to fluctuations in IKK, is much larger than σ_{int} except for large times (13 hours) after IKK activation. Thus for most of the time after IKK activation, extrinsic fluctuations dominate. The magnitude of σ_{tot} shortly after IKK activation reflects the significance of IKK fluctuations in determining cell-to-cell variability in terms of both amplitude and period of $NF-\kappa B$ oscillations.

In this subsection, we have studied the effect of varying the initial amount of activated IKK around an average value, which we denote by IKK_0 , while keeping the number of $NF-\kappa B$ molecules fixed. Let us now comment on how average oscillations change when IKK_0 changes significantly compared to the value considered up to now. When IKK_0 decreases by 50% compared to the value chosen by Hoffmann et al. (2002), oscillations for both linear and quadratic transcription remain strong with an average increase in period of 20 to 30 percent; for an increase of 50% in IKK_0 , the period of oscillations decreases for quadratic transcription, whereas for linear transcription oscillations are significantly damped beyond the first one. In the experiments discussed (Hoffmann et al., 2002; Nelson et al., 2004) there are no results for the effect of varying the amount of the external stimulus, $TNF\alpha$, on $NF-\kappa B$ oscillations. We note that the utility of our predictions for the average oscillations as a function of activated IKK depends on knowing how the amount of activated IKK is related to the amount of external stimulant.

4 Discussion.

The data of Hoffmann et al. (2002) and Nelson et al. (2004) on $NF-\kappa B$ oscillations are seemingly contradictory. Hoffmann et al. (2002, suppl. material, fig. S1) observe well-defined oscillations for a population of cells, whereas Nelson et al. (2004) observe (for the identical stimulus) large cell-to-cell variability, in both amplitude and period of oscillations, which would normally lead to washed out average oscillations. However both groups use different cell lines, and experimental methods differ (for a discussion of these see the technical comment of Barken et al. (2005) and the response by Nelson et al. (2005)).

From a modeling point of view, for the class of reactions considered here (cf. Table 1), we summarize our key results:

- *intrinsic* fluctuations are small when transcription is strong (which is the case of the model of Hoffmann et al.(2002)), whether transcription is linear or quadratic in the number of $NF-\kappa B$ molecules. However intrinsic fluctuations can be strong, as significant as any extrinsic noise,

if the rate of promoter binding is small, and rate constants for transcription and translation of $I\kappa B$ become very different from the ones used by Hoffmann et al. (2002); if this is the case intrinsic noise can account for the observed cell-to-cell variability. Hence, it is important to determine transcription rate constants experimentally.

In all cases considered, *extrinsic* fluctuations can be significant:

- in the model of Hoffmann et al. (2002), fluctuations in the amount of activated IKK molecules can account for the observed cell-to-cell variability (Nelson et al., 2004), because they affect both amplitude and period of $NF-\kappa B$ oscillations, whatever the mode of transcription, linear or quadratic in the number of $NF-\kappa B$ molecules, of $I\kappa B\alpha$.

- as to fluctuations in the amount of $NF-\kappa B$ molecules, their impact depends on the type of transcription, linear or quadratic. For linear transcription the amplitude only of oscillations varies, not their periodicity, whereas for quadratic transcription, both amplitude and periodicity vary. Only in the latter case would these fluctuations be a possible source of the observed cell-to-cell variability (Nelson et al., 2004)

Fluctuations in individual cell behavior thus provide a source of damping for $NF-\kappa B$ population oscillations that is different from the one considered by Hoffmann et al. (2002), which depends on the existence of $I\kappa B$ isoforms besides $I\kappa B\alpha$.

Our results on cell-to-cell variability of $NF-\kappa B$ oscillations (in the context of the model of Hoffmann et al., 2002) help clarify the experimental issues that need to be resolved for determining its origins. These requirements can be formulated as questions: what is the distribution over the cell population of the amount of $NF-\kappa B$, and of activated IKK ; what are the rates of transcription and translation; is transcription linear or quadratic (in the right range) in the number of $NF-\kappa B$ molecules? When answers to these questions will be forthcoming, one will be able to determine, within a given model, the sources of cell-to-cell variability of $NF-\kappa B$ oscillations, and eventually reconcile the well-defined average oscillations observed by Hoffmann et al. (2002) and the large cell-to-cell variability of Nelson et al. (2004) as a matter of differences in the amount of fluctuations due to cell type, cell environment or experimental method.

The key biological issue is the functional role of $NF-\kappa B$ oscillations, which is not known. Since $NF-\kappa B$ participates in regulating the expression of many genes, its oscillations can be transferred to the corresponding proteins. One can speculate that the number of $NF-\kappa B$ oscillations in a given time period could play the role of a threshold for setting into motion many aspects of cellular response. Consider Figure 8: as the level of IKK goes down the period of oscillations lengthens. Assume that four oscillations in the first 400 minutes after IKK activation represent the threshold below which signaling based on $NF-\kappa B$ oscillations becomes ineffective. In Figure 8, the case $IKK=25000$ is marginal ($NF-\kappa B$ is fixed). Thus as the amount of IKK goes lower, say to $IKK=20000$, and if this amount represents the average amount of IKK activated by an outside agent, only fluctuations in IKK will enable

some cells to reach the threshold of four oscillations in the required time, and thus respond to the external stimulation. This effect of stochasticity would lead to cellular response even for small, below threshold, doses of external agent, as might happen for immune response to a pathogen.

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Table 1: Reactions and Rate constants

| Rate constant | Reaction | Value |
|---------------|------------------------------|--------------------------|
| a_4 | $Ic + Nc \rightarrow INc$ | $30\mu M^{-1}min^{-1}$ |
| d_4 | $INc \rightarrow Ic + Nc$ | $0.03min^{-1}$ |
| a_7 | $IKK + INc \rightarrow INcp$ | $11.1\mu M^{-1}min^{-1}$ |
| d_1 | $INcp \rightarrow IKK + INc$ | $0.075min^{-1}$ |
| r_4 | $INcp \rightarrow IKK + Nc$ | $1.221min^{-1}$ |
| a_1 | $IKK + Ic \rightarrow Icpr$ | $1.35\mu M^{-1}min^{-1}$ |
| d_1 | $Icpr \rightarrow IKK + Ic$ | $0.075min^{-1}$ |
| a_4 | $Icpr + Nc \rightarrow INcp$ | $30\mu M^{-1}min^{-1}$ |
| d_4 | $INcp \rightarrow Icpr + Nc$ | $0.03min^{-1}$ |
| r_1 | $Icpr \rightarrow IKK$ | $0.2442min^{-1}$ |
| deg_1 | $Ic \rightarrow \emptyset$ | $0.00675min^{-1}$ |
| tp_1 | $Ic \rightarrow In$ | $0.018min^{-1}$ |
| tp_2 | $In \rightarrow Ic$ | $0.012min^{-1}$ |
| k_1 | $Nc \rightarrow Nn$ | $5.4min^{-1}$ |
| k_{01} | $Nn \rightarrow Nc$ | $0.0048min^{-1}$ |
| k_2 | $INn \rightarrow INc$ | $0.8294min^{-1}$ |
| tr_2 | $Nn \rightarrow Nn + Ictr$ | $0.0582min^{-1}$ |
| tr_3 | $Ictr \rightarrow \emptyset$ | $0.0168min^{-1}$ |
| tr_1 | $Ictr \rightarrow Ictr + Ic$ | $0.2448min^{-1}$ |
| a_4 | $In + Nn \rightarrow INn$ | $30\mu M^{-1}min^{-1}$ |
| d_4 | $INn \rightarrow In + Nn$ | $0.03min^{-1}$ |
| k_{02} | $IKK \rightarrow \emptyset$ | $0.0072min^{-1}$ |

Rate constants are from Nelson et al. (2004), based on the model of Hoffmann et al. (2002).

Notation:

Nn= nuclear $NF-\kappa B$, Nc= cytoplasmic $NF-\kappa B$

In= nuclear $I\kappa B\alpha$, Ic=cytoplasmic $I\kappa B\alpha$

INc= cytoplasmic $NF-\kappa B-I\kappa B\alpha$ complex

INn= nuclear $NF-\kappa B-I\kappa B\alpha$ complex

INcp= complex of IKK and INc

Icpr= complex of IKK and $I\kappa B\alpha$

Ictr= $I\kappa B\alpha$ mRNA

6 Figure captions

Figure 1. Nuclear $NF-\kappa B$ as a function of time for the model with linear transcription. IKK activation sets in after 3000 minutes. Initial total amount of $NF-\kappa B$ and amount of IKK are fixed at $0.05 \mu M$. The full curve is average behavior, the broken curves show three randomly chosen single cell oscillations.

Figure 2. Nuclear $NF-\kappa B$ as a function of time for the model with linear transcription. Here the values of rate constants $tr2$ for transcription and $tr1$ for translation are $1/10$ and 10 times respectively the values given in the Table, which are used for Figure 1 (see text). IKK activation sets in after 3000 minutes. Initial total amount of $NF-\kappa B$ and amount of IKK are fixed at $0.05 \mu M$. The full and two broken curves correspond to three randomly chosen single cell oscillations and show the increased cell-to-cell variability as compared to Figure 1.

Figure 3. Nuclear $NF-\kappa B$ as a function of time for the model variant described in section 3.1.1. Here $kb = 1200 min^{-1}$. IKK activation sets in after 3000 minutes. Initial total amount of $NF-\kappa B$ and amount of IKK are fixed at $0.05 \mu M$. The full curve is the average behavior of $NF-\kappa B$, the broken curve represents the standard deviation due to intrinsic noise.

Figure 4. Nuclear $NF-\kappa B$ as a function of time for the model variant described in section 3.1.1. Here $kb = 12 min^{-1}$; transcription is weak. The full curve is average cell behavior, the broken line is the standard deviation σ .

Figure 5. Nuclear $NF-\kappa B$ as a function of time for the model variant described in section 3.1.1. Here $kb = 12 min^{-1}$; transcription is weak. The curves correspond to the behavior of three single cells. Oscillations differ in both amplitude and phase.

Figure 6. Nuclear $NF-\kappa B$ as a function of time for the model with linear transcription. IKK activation sets in after 3000 minutes. The amount of initially activated IKK is $0.05 \mu M$. The full curve represents an average over 1000 single cells for an initial amount of $NF-\kappa B$ following a gaussian distribution of average number equal to 30000 (corresponding to $0.05 \mu M$ for the cell volume chosen), and width $\sigma = 5000$. The broken curves correspond to one single cell behavior for, by decreasing amplitude, $NF-\kappa B = 35000$, 25000 , and 20000 molecules.

Figure 7. Nuclear $NF-\kappa B$ as a function of time for the model with quadratic transcription. IKK activation sets in after 3000 minutes. The amount of initial activated IKK is $0.05 \mu M$. The full curve represents an average over 1000 single cells for an initial amount of $NF-\kappa B$ following a Gaussian distribution of average number equal to 30000 (corresponding to $0.05 \mu M$ for the cell volume chosen), and width $\sigma = 5000$.

Figure 8. Nuclear $NF-\kappa B$ as a function of time for the model with linear transcription. IKK activation sets in after 3000 minutes. The amount of initial $NF-\kappa B$ is fixed at $0.05 \mu M$. The full curve represents an average over 1000 single cells for an initial amount of IKK following a Gaussian distribution of average number equal to 30000 (corresponding to 0.05

μM for the cell volume chosen), and width $\sigma = 5000$. The broken curves correspond to single cell behavior for, by decreasing amplitude, IKK= 25000 and 35000 molecules.

Figure 9. Standard deviations (in μM) of nuclear $NF-\kappa B$ oscillations for the case considered in Figure 3, as a function of time. IKK activation sets in after 3000 minutes. σ_{tot} (broken line) represents the total noise, σ_{int} (full line) the intrinsic noise (see text).

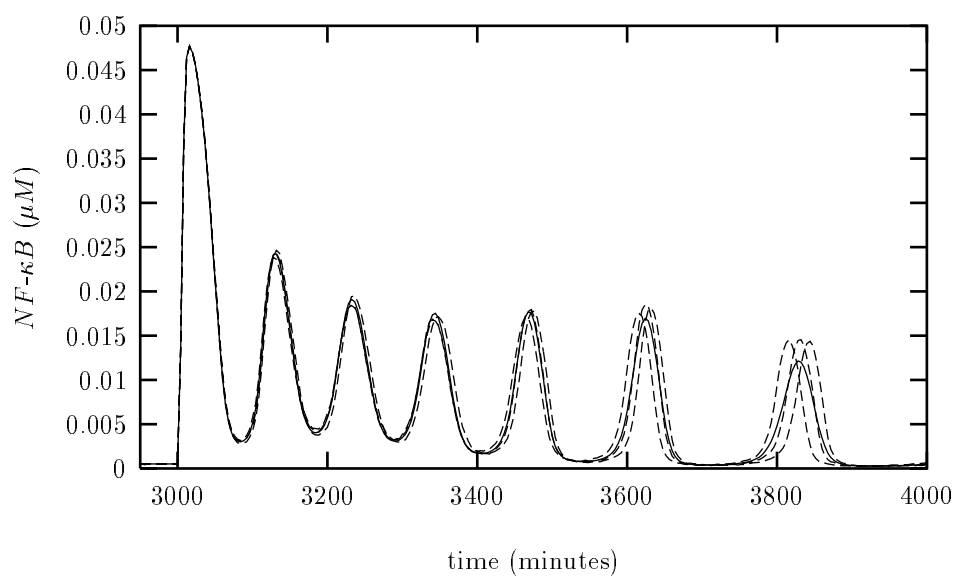


Figure 1:

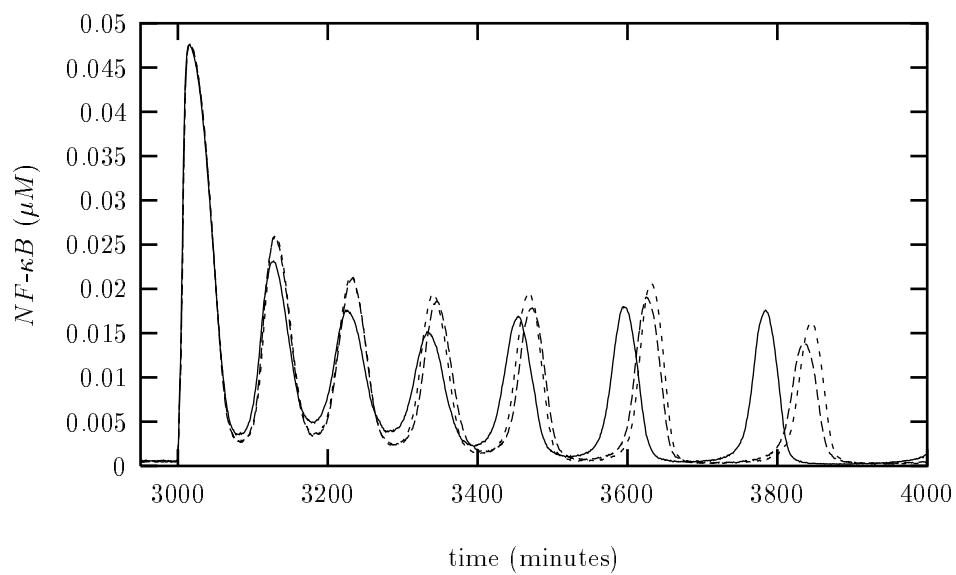


Figure 2:

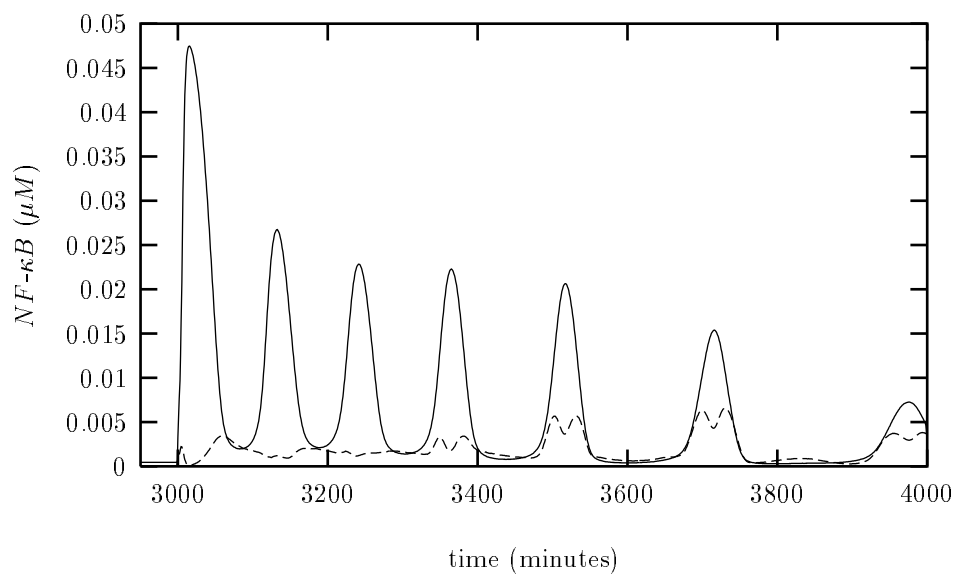


Figure 3:

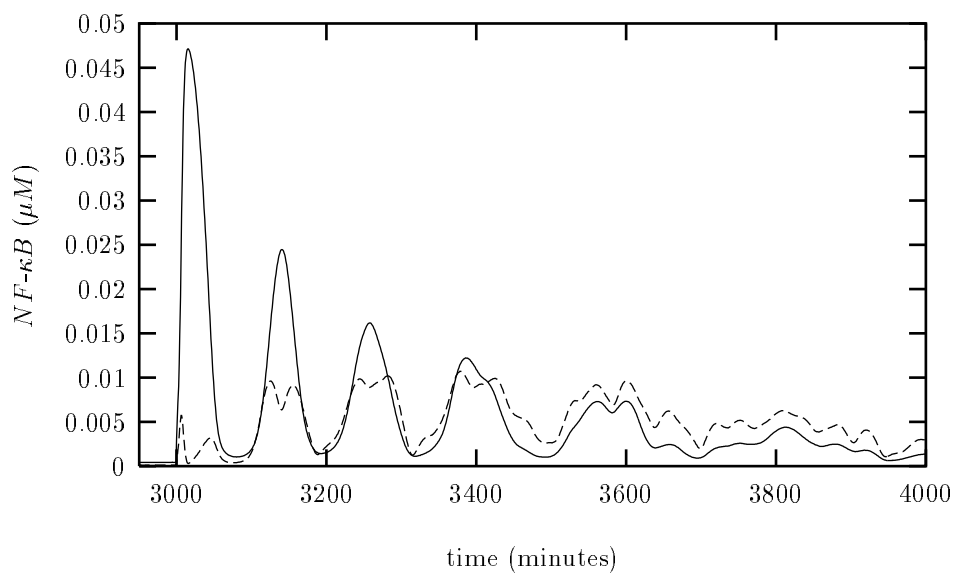


Figure 4:

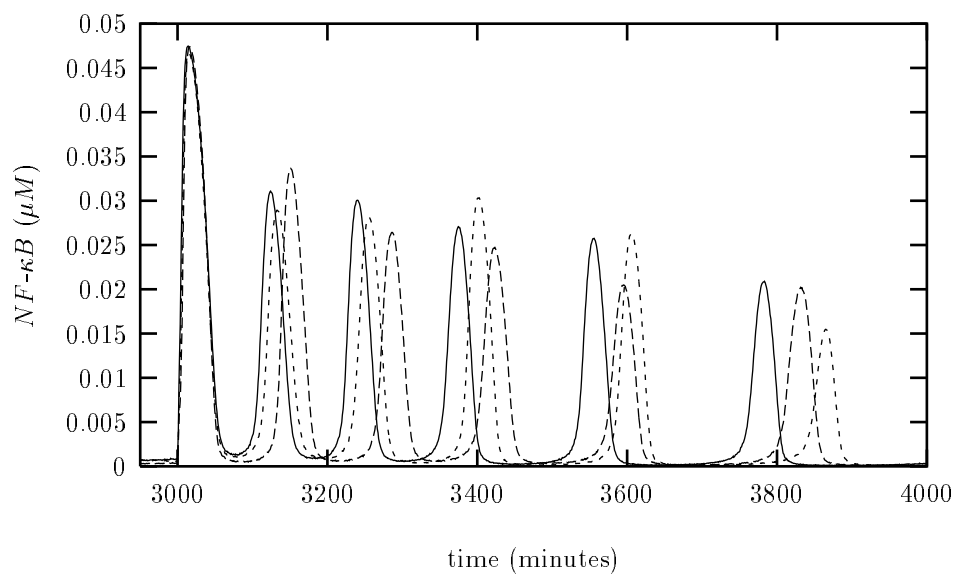


Figure 5:

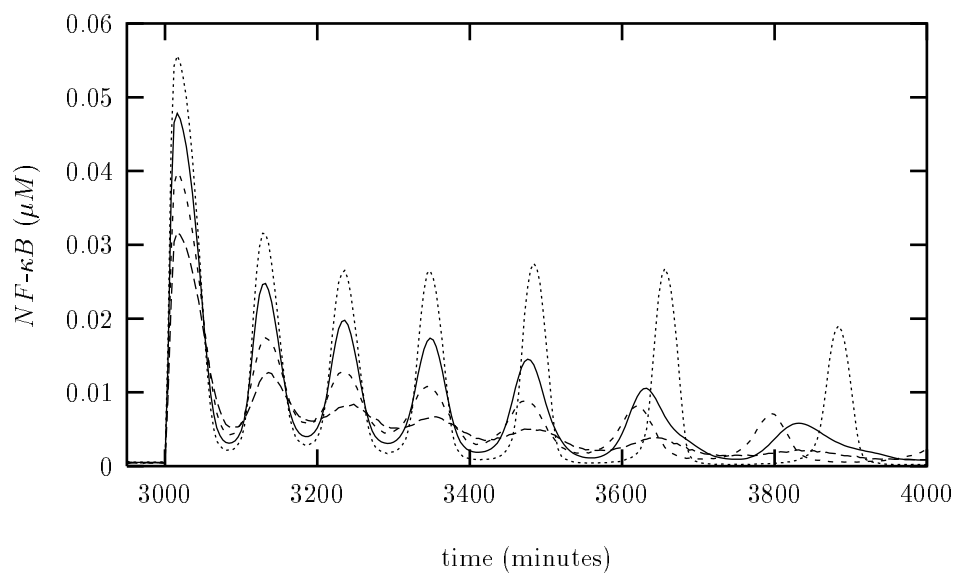


Figure 6:

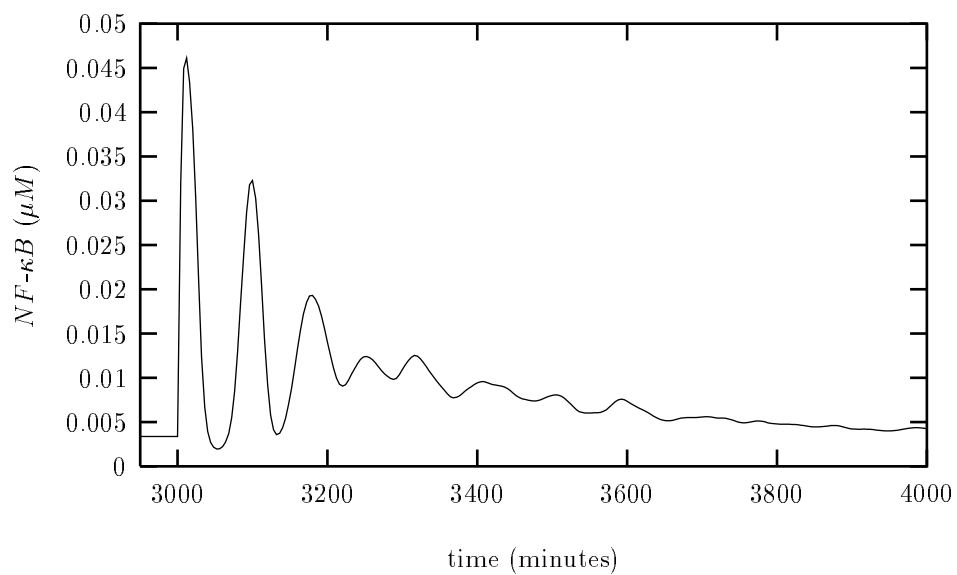


Figure 7:

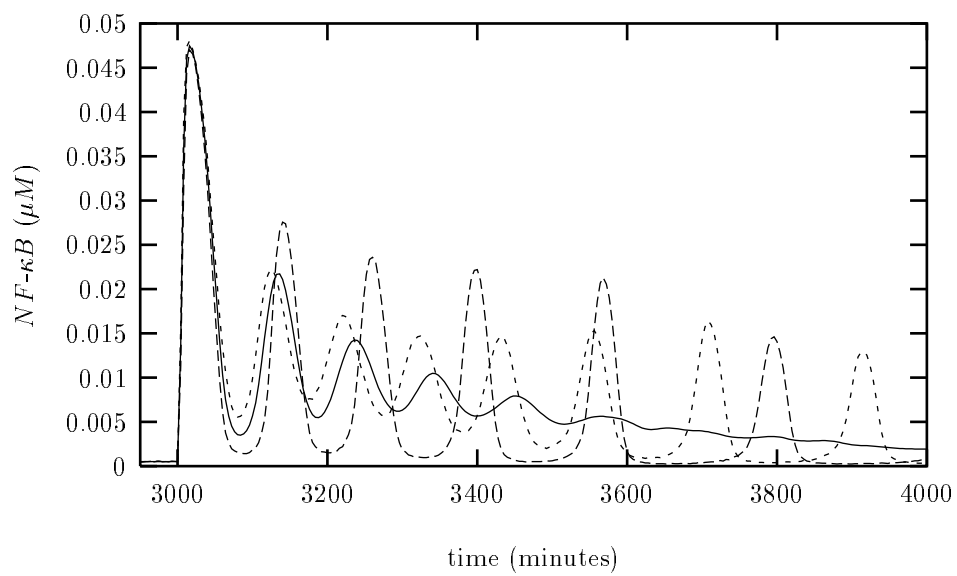


Figure 8:

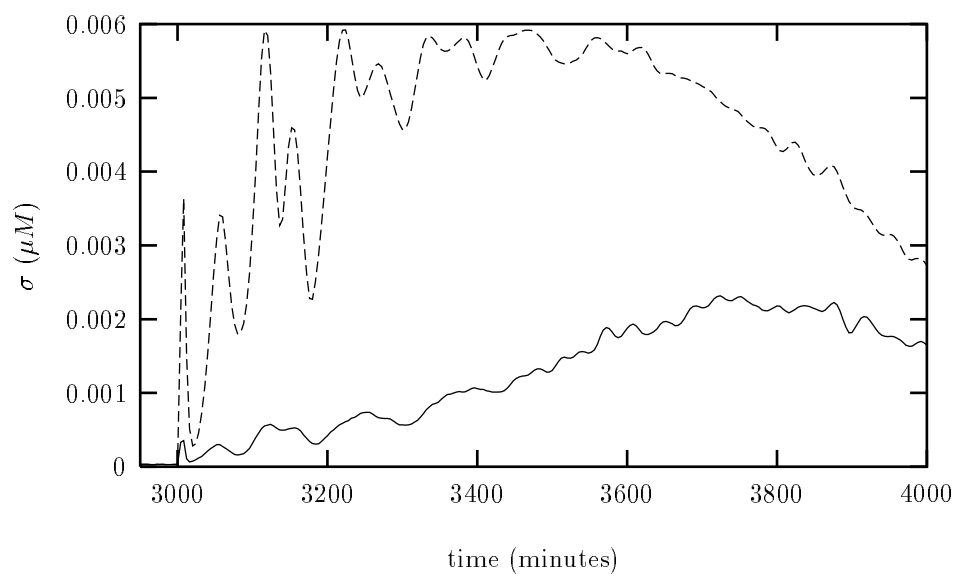


Figure 9: